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SECOND ADDENDUM
TO THE
BRITISH PHARMACOPŒIA
1932

PUBLISHED UNDER THE DIRECTION OF

THE GENERAL COUNCIL OF
MEDICAL EDUCATION AND REGISTRATION
OF THE UNITED KINGDOM

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NOTICE

By Section 2 of the Medical Council Act, 1862, the exclusive right of publishing, printing, and selling the British Pharmacopœia is vested in the General Council of Medical Education and Registration of the United Kingdom.

The British Pharmacopœia, 1932, superseded previous issues of the British Pharmacopœia, being for all purposes deemed to be substituted for such previous issues.

The Addendum, 1936, altered and amended the British Pharmacopœia, 1932, and this Second Addendum effects further alterations and emendations. The General Notices and Appendices included in the British Pharmacopœia, 1932, and the Addendum, 1936, apply to all matter contained in this Addendum, unless the contrary is specifically stated.

This Addendum has the same authority as the British Pharmacopœia, 1932, as amended by the Addendum, 1936. Monographs of the British Pharmacopœia, 1932, or of the Addendum, 1936, which are amended by this Addendum supersede, in their amended forms, the original monographs.

PREFACE

TO THE SECOND ADDENDUM TO THE BRITISH PHARMACOPŒIA, 1932

SECTION 54 of the Medical Act, 1858, provides that the General Council of Medical Education and Registration of the United Kingdom 'shall cause to be published under their direction a Book containing a list of medicines and compounds, and the manner of preparing them, together with the true weights and measures by which they are to be prepared and mixed, and containing such other matter and things relating thereto as the General Council shall think fit, to be called "The British Pharmacopœia"; and the General Council shall cause to be altered, amended, and republished, such Pharmacopœia as often as they shall deem it necessary.'

This Addendum to the British Pharmacopœia, 1932, has been prepared by the British Pharmacopœia Commission and approved by the Pharmacopœia Committee of the Council in the discharge of the duty entrusted to them by the Standing Orders of the Council to deal with all matters relating to the preparation and publication of the British Pharmacopœia.

The Pharmacopœia Committee of the Council, in a Report made by it to the Council in accordance with the Standing Orders, has conveyed to the Council a cordial expression of its appreciation of the work done by the Commission in preparing this Addendum; and also by the persons and bodies, both in this country and abroad, by whose collaboration that task has been facilitated.

GENERAL MEDICAL COUNCIL OFFICE,
44 HALLAM STREET, PORTLAND PLACE,
LONDON, W.1.

THE
BRITISH
PHARMACOPŒIA COMMISSION

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* Dr. A. P. BEDDARD, Consulting Physician to Guy's Hospital, was Chairman of the Commission until his death in November 1939.

Additions to the British Pharmacopœia, 1932

- Emulsio Olei Morrhuæ
- Emulsio Olei Vitaminati
- Extractum Malti cum Oleo Vitaminato
- Liquor Vitamini A Concentratus
- Liquor Vitamini D Concentratus
- Liquor Vitaminorum A et D Concentratus
- Oleum Amygdalæ Volatile Purificatum
- Oleum Hippoglossi
- Oleum Vitaminatum
- Toxinum Tetanicum Detoxicatum

Monographs of the British Pharmacopœia, 1932, which are amended by the Second Addendum

- Linimentum Camphoræ
- Unguentum Acidi Tannici
- Unguentum Aquosum
- Unguentum Capsici
- Unguentum Hydrargyri Compositum

Appendices to the British Pharmacopœia, 1932, and the Addendum, 1936, which are amended by the Second Addendum

Appendix IV. G.	Determination of Ultra-violet Absorption
Appendix X. C.	Determination of Iodine Value
Appendix X. D.	Determination of the Unsaponifiable Matter in Fixed Oils and Fats
Appendix XI. C.	Determination of Aldehydes in Volatile Oils
Appendix XV. O.	Assay of Vitamin A

MONOGRAPHS

EMULSIO OLEI MORRHUÆ

[Emuls. Ol. Morrh.]

Emulsion of Cod-liver Oil

Cod-liver Oil	500	millilitres
Acacia, in powder . .	125	grammes
Tragacanth, in powder .	7	grammes
Purified Volatile Oil of Bitter Almond	1	millilitre
Soluble Saccharin . .	0·1	gramme
Chloroform	2	millilitres
Distilled Water, sufficient to produce	1000	millilitres

Triturate quickly the Cod-liver Oil with the Acacia and Tragacanth; without delay add in one quantity 250 millilitres of Distilled Water and stir briskly until emulsified. Add the Soluble Saccharin, dissolved in 2 millilitres of Distilled Water, the Purified Volatile Oil of Bitter Almond, the Chloroform and sufficient Distilled Water to produce the required volume.

If emulsification is effected by mechanical means, the quantities of Tragacanth and Acacia may be reduced at the discretion of the operator, provided that the proportions of the other ingredients are not changed and the viscosity of the resulting emulsion is the same.

DOSES

Metric.	**Imperial.**
Prophylactic.	
2 to 4 mils.	**30 to 60 minims.**

Approximately equivalent doses in Units.

Vitamin A	**1000 to 2000 Units.**
Vitamin D	**100 to 200 Units.**

Therapeutic.

6 to 12 mils.	**90 to 180 minims.**

Approximately equivalent doses in Units.

Vitamin A 3000 to 6000 Units.
Vitamin D 300 to 600 Units.

EMULSIO OLEI VITAMINATI

[Emuls. Ol. Vitamin.]

Emulsion of Vitaminised Oil

Vitaminised Oil	500	millilitres
Acacia, in powder	125	grammes
Tragacanth, in powder	7	grammes
Purified Volatile Oil of Bitter Almond	1	millilitre
Soluble Saccharin	0·1	gramme
Chloroform	2	millilitres
Distilled Water, sufficient to produce	1000	millilitres

Triturate quickly the Vitaminised Oil with the Acacia and Tragacanth; without delay add in one quantity 250 millilitres of Distilled Water and stir briskly until emulsified. Add the Soluble Saccharin, dissolved in 2 millilitres of Distilled Water, the Purified Volatile Oil of Bitter Almond, the Chloroform and sufficient Distilled Water to produce the required volume.

If emulsification is effected by mechanical means, the quantities of Tragacanth and Acacia may be reduced at the discretion of the operator, provided that the proportions of the other ingredients are not changed and the viscosity of the resulting emulsion is the same.

DOSES

Metric.	**Imperial.**
Prophylactic.	
2 to 4 mils.	**30 to 60 minims.**

Approximately equivalent doses in Units.

Vitamin A 1000 to 2000 Units.
Vitamin D 100 to 200 Units.

Therapeutic.

6 to 12 mils.	90 to 180 minims.

Approximately equivalent doses in Units.

Vitamin A	3000 to 6000 Units.
Vitamin D	300 to 600 Units.

EXTRACTUM MALTI CUM OLEO VITAMINATO

[Ext. Malt. c. Ol. Vitamin.]

Extract of Malt with Vitaminised Oil

Extract of Malt with Vitaminised Oil contains approximately 15 per cent. v/v of Vitaminised Oil.

Extract of Malt . . .	900 grammes
Vitaminised Oil . . .	100 grammes

Mix thoroughly with the aid of gentle heat.

DOSES

Metric.	Imperial.
4 to 16 mils.	60 to 240 minims.

Approximately equivalent doses in Units.

Vitamin A	650 to 2500 Units.
Vitamin D	65 to 250 Units.

Extract of Malt with Vitaminised Oil contains in 16 mils about 2·5 mils and in 240 minims about 36 minims, of Vitaminised Oil.

LINIMENTUM CAMPHORÆ

[Lin. Camph.]

Liniment of Camphor

Synonym. Camphorated Oil.

Arachis Oil, Cottonseed Oil or Sesame Oil may be used, in place of Olive Oil, in making this Liniment.

LIQUOR VITAMINI A CONCENTRATUS

[Liq. Vitamin. A Conc.]

Concentrated Solution of Vitamin A

Concentrated Solution of Vitamin A is a solution of Vitamin A containing in 1 gramme 50,000 *Units of Vitamin A activity*. It may consist of a suitable fish-liver oil or blend of fish-liver oils, or it may be prepared by dissolving, at a temperature not exceeding 60°, a source of vitamin A in a suitable vegetable oil, such as Arachis Oil. As the source of vitamin A any of the following materials, or any suitable mixture of them, may be used :—a fish oil rich in vitamin A ; the unsaponifiable matter, separated from the glycerides, of such oil ; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter, or by any other suitable method.

Characters. A pale yellow, or yellow, oily liquid ; odour, faint and not rancid ; taste, bland, or slightly fishy.

Slightly soluble in *alcohol* (*95 per cent.*) ; miscible with *ether*, with *chloroform*, and with *light petroleum* (*boiling-point, 50° to 60°*).

Tests for Purity. *Acid value*, not greater than 2·5 ; *antirachitic activity* (*vitamin D*), not more than 500 Units per gramme.

Assay. Determine the vitamin A activity in relation to the Standard Preparation of vitamin A by the *assay of vitamin A*, and express the result in Units per gramme.

Storage. Concentrated Solution of Vitamin A should be kept in a well-filled, well-closed container, and protected from light.

DOSES

Metric.	Imperial.
0·06 to 0·3 mil.	1 to 5 minims.

Approximately equivalent doses in Units.

Vitamin A 2500 to 12,500 Units.

LIQUOR VITAMINI D CONCENTRATUS

[Liq. Vitamin. D Conc.]

Concentrated Solution of Vitamin D

Concentrated Solution of Vitamin D is a solution of vitamin D containing in 1 gramme 10,000 *Units of antirachitic activity* (*vitamin D*). It may consist of a suitable fish-liver oil or blend of fish-liver oils, or it may be prepared by dissolving, at a temperature not exceeding 60°, a source of vitamin D in a suitable vegetable oil, such as Arachis Oil. As the source of vitamin D any of the following materials, or any suitable mixture of them, may be used:—Calciferol; a fish oil rich in vitamin D; the unsaponifiable matter, separated from the glycerides, of such oil; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter; any other source of the antirachitic substance found in fish livers.

Characters. A pale yellow, or yellow, oily liquid; odour, faint and not rancid; taste, bland, or slightly fishy.

Slightly soluble in *alcohol* (*95 per cent.*); miscible with *ether*, with *chloroform*, and with *light petroleum* (*boiling-point, 50° to 60°*).

Tests for Purity. *Acid value*, not greater than 2·5; *vitamin A activity*, not more than 5000 Units per gramme.

Assay. Determine the antirachitic activity in relation to the Standard Preparation of antirachitic vitamin (vitamin D) by the *biological assay of antirachitic vitamin* (*vitamin D*) and express the result in Units per gramme.

Storage. Concentrated Solution of Vitamin D should be kept in a well-filled, well-closed container, and protected from light.

DOSES
daily

Metric.	**Imperial.**
0·03 to 0·2 mil.	**$^{1}/_{2}$ to 3 minims.**

Approximately equivalent doses in Units.

Vitamin D 250 to 1500 Units.

LIQUOR VITAMINORUM A ET D CONCENTRATUS

[Liq. Vitamin. A et D Conc.]

Concentrated Solution of Vitamins A and D

Concentrated Solution of Vitamins A and D is a solution containing in 1 gramme 50,000 *Units of Vitamin A activity*, and 5000 *Units of antirachitic activity* (*vitamin D*). It may consist of a suitable fish-liver oil or blend of fish-liver oils, or it may be prepared by dissolving, at a temperature not exceeding 60°, a source of vitamin A and a source of vitamin D in a suitable vegetable oil, such as Arachis Oil. As the source of vitamin A any of the following materials, or any suitable mixture of them, may be used:—a fish oil rich in vitamin A; the unsaponifiable matter, separated from the glycerides, of such oil; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter, or by any other suitable method. As the source of vitamin D any of the following materials, or any suitable mixture of them, may be used:—Calciferol; a fish oil rich in vitamin D; the unsaponifiable matter, separated from the glycerides, of such oil; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter; any other source of the antirachitic substance found in fish livers.

Characters. A pale yellow, or yellow, oily liquid; odour, faint and not rancid; taste, bland, or slightly fishy.

Slightly soluble in *alcohol* (*95 per cent.*); miscible with *ether*, with *chloroform*, and with *light petroleum* (*boiling-point, 50° to 60°*).

Test for Purity. *Acid value*, not greater than 2·5.

Assay. *For vitamin A activity.* Determine the vitamin A activity in relation to the Standard Preparation of vitamin A by the *assay of vitamin A* and express the result in Units per gramme.

For antirachitic activity (*vitamin D*). Determine the antirachitic activity in relation to the Standard Preparation of antirachitic vitamin (vitamin D) by the *biological assay of antirachitic vitamin* (*vitamin D*) and express the result in Units per

Storage. Concentrated Solution of Vitamins A and D should be kept in a well-filled, well-closed container, and protected from light.

DOSES

Metric.	**Imperial.**
0·06 to 0·3 mil.	**1 to 5 minims.**

Approximately equivalent doses in Units.

Vitamin A	**2500 to 12,500**	**Units.**
Vitamin D	**250 to 1250**	**Units.**

OLEUM AMYGDALÆ VOLATILE PURIFICATUM

[Ol. Amygdal. Vol. Purif.]

Purified Volatile Oil of Bitter Almond

Purified Volatile Oil of Bitter Almond is prepared from the cake left after pressing out the fixed oil from bitter almonds, peach kernels, or apricot kernels, by distillation with water and purification of the resulting distillate by the removal of hydrocyanic acid. It contains not less than 95 per cent. of benzaldehyde, C_6H_5CHO.

Characters. A colourless, or pale yellow, liquid; odour and taste, that of bitter almonds.

Tests for Identity and Purity. Soluble in 2 parts of *alcohol (70 per cent.)*; *specific gravity (15·5°/15·5°)*, 1·048 to 1·052; *refractive index* at 20°, 1·542 to 1·546.

Shake 1 millilitre with 1 millilitre of *N/1 sodium hydroxide*, add 0·5 millilitre of *solution of ferrous sulphate*, warm gently, again shake and acidify with *dilute hydrochloric acid*; no blue colour is produced (absence of hydrocyanic acid).

Heat a closely wound coil, 6 millimetres in diameter and 6 millimetres long, of copper wire, about 0·06 millimetre in diameter, in a bunsen flame until it ceases to colour the flame green; cool, dip in the oil, ignite and allow to burn outside the bunsen flame and then bring the coil into contact with the edge of the flame; no transient green colour is imparted to the flame (limit of chlorinated compounds).

Assay. Carry out the *method for the determination of aldehydes in volatile oils, Purified Volatile Oil of Bitter Almond.*

Storage. Purified Volatile Oil of Bitter Almond should be kept in a well-closed container, protected from light, and stored in a cool place.

DOSES

Metric.	**Imperial.**
0·016 to 0·06 mil.	**$^1/_4$ to 1 minim.**

OLEUM HIPPOGLOSSI

[Ol. Hippogloss.]

Halibut-liver Oil

Halibut-liver Oil is the fixed oil extracted from the fresh, or suitably preserved, liver of the halibut, *Hippoglossus vulgaris*. Halibut-liver Oil contains in each gramme not less than 30,000 *Units of vitamin A activity*.

Characters. A pale to golden-yellow liquid; odour, fishy, but not rancid; taste, fishy.

Slightly soluble in *alcohol* (*90 per cent.*); miscible with *ether*, with *chloroform*, and with *light petroleum* (*boiling-point, 50° to 60°*).

Tests for Purity. *Specific gravity* (*15·5°/15·5°*), 0·924 to 0·929; *acid value*, not greater than 1·0; *saponification value*, not greater than 175; *iodine value* (*pyridine bromide method*), not less than 115; *iodine value of glycerides*, 115 to 130; *unsaponifiable matter*, not less than 8 per cent.

Ultra-violet absorption at 300mμ not greater than 75 per cent. of that at 328mμ (absence of whale-liver oil).

Assay. Determine the vitamin A activity by the *assay of vitamin A*.

Storage. Halibut-liver Oil should be kept in a well-filled, well-closed container, and protected from light.

DOSES

Metric.	Imperial.
0·06 to 0·3 mil.	1 to 5 minims.

Approximately equivalent doses in Units.

Vitamin A 1500 to 7500 Units.

The vitamin D activity of Halibut-liver Oil varies; it is usually between 2500 and 3500 Units per gramme. When a statement is made of the number of Units of vitamin D in Halibut-liver Oil, the Units enumerated should be the Units described under the *biological assay of antirachitic vitamin* (*vitamin D*).

OLEUM VITAMINATUM

[Ol. Vitamin.]

Vitaminised Oil

Vitaminised Oil is a solution of vitamins A and D containing in 1 gramme 1000 *Units of vitamin A activity* and 100 *Units of antirachitic activity* (*vitamin D*). It may consist of a suitable fish-liver oil, or blend of fish-liver oils, or it may be prepared by dissolving, at a temperature not exceeding 60°, a source of vitamin A and a source of vitamin D in a suitable vegetable oil, such as Arachis Oil. As the source of vitamin A any of the following materials, or any suitable mixture of them, may be used :—Concentrated Solution of Vitamin A ; Concentrated Solution of Vitamins A and D ; a fish oil rich in vitamin A ; the unsaponifiable matter, separated from the glycerides, of such oil ; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter, or by any other suitable method. As the source of vitamin D any of the following materials, or any suitable mixture of them, may be used :—Concentrated Solution of Vitamin D ; Concentrated Solution of Vitamins A and D ; Calciferol ; a fish oil rich in vitamin D ; the unsaponifiable matter, separated from the glycerides, of such oil ; a concentrate prepared by partial saponification, distillation or extraction of such oil, or by distillation or extraction of such unsaponifiable matter ; any other source of the antirachitic substance found in fish livers.

Characters. A pale yellow, or yellow, oily liquid ; odour, faint and not rancid ; taste, bland, or slightly fishy.

Slightly soluble in *alcohol* (*95 per cent.*) ; miscible with *ether*, with *chloroform*, and with *light petroleum* (*boiling-point, 50° to 60°*).

Tests for Purity. *Acid value*, not greater than 2·5 ; *unsaponifiable matter*, not more than 2 per cent.

Assay. *For vitamin A activity.* Determine the vitamin A activity in relation to the Standard Preparation of vitamin A by the *assay of vitamin A* and express the result in Units per gramme.

For antirachitic activity (*vitamin D*). Determine the antirachitic activity in relation to the Standard Preparation of antirachitic vitamin (vitamin D) by the *biological assay of antirachitic vitamin (vitamin D)* and express the result in Units per gramme.

Storage. Vitaminised Oil should be kept in a well-filled, well-closed container, protected from light, and stored in a cool place.

DOSES

Metric.	**Imperial.**
Prophylactic.	
1 to 2 mils.	15 to 30 minims.

Approximately equivalent doses in Units.

Vitamin A 1000 to 2000 Units.
Vitamin D 100 to 200 Units.

Metric.	**Imperial.**
Therapeutic.	
3 to 6 mils.	45 to 90 minims.

Approximately equivalent doses in Units.

Vitamin A 3000 to 6000 Units.
Vitamin D 300 to 600 Units.

TOXINUM TETANICUM DETOXICATUM

[Toxin. Tetanic. Detoxicat.]

Tetanus Toxoid

CAUTION—In any part of the British Empire in which Tetanus Toxoid is controlled by law, care must be taken that the provisions of such law are duly complied with. (See British Pharmacopœia, 1932, page 12.)

Tetanus Toxoid is tetanus toxin, the sterile filtrate of a culture on a suitable medium of *Clostridium tetani*, or material derived therefrom, the specific toxicity of which has been completely removed by the action of chemical substances in such a manner that it retains efficient properties as an immunising antigen.

Tetanus Toxoid may occur in the following forms:—(*a*) Tetanus Toxoid in simple solution, (*b*) Alum Precipitated

Tetanus Toxoid. Tetanus Toxoid in simple solution is prepared by treating the filtrate with formaldehyde. Alum Precipitated Tetanus Toxoid is prepared by adding Alum to Tetanus Toxoid in simple solution in the proportion necessary to produce a suitable precipitate, separating the precipitate, and washing and suspending it in Physiological Solution of Sodium Chloride.

Characters. Tetanus Toxoid in simple solution is a clear, yellow or colourless liquid, free from particles. Alum Precipitated Tetanus Toxoid is a suspension of white, slightly yellow or yellowish-brown particles in a colourless liquid.

Test for Identity. When injected into animals it produces tetanus antitoxin in the circulating blood, or increases the amount of this antitoxin, if already present.

Test for Purity. Complies with the *tests for sterility.*

Test for Absence of Specific Toxicity. 5 millilitres of Tetanus Toxoid in simple solution, or 1 millilitre of Alum Precipitated Tetanus Toxoid, injected subcutaneously into each of not fewer than five normal guinea-pigs, each weighing from 250 to 350 grammes, does not produce any symptom of tetanus during the following twenty-one days in any of the animals injected.

Test for Potency as an Immunising Antigen. Not fewer than nine normal guinea-pigs, each weighing from 250 to 350 grammes, are used for the test. Each guinea-pig receives the tetanus toxoid by subcutaneous injection, either in a single dose of 5 millilitres of tetanus toxoid in simple solution or of 1 millilitre of alum-precipitated tetanus toxoid, or in two doses, each of 0·1 millilitre of either form, separated by an interval of not more than four weeks; or some may receive the single dose and the remainder may receive the two doses. Not later than six weeks after the single injection, or, if two injections have been given, not later than two weeks after the second injection, the serum of each of not fewer than two-thirds of the guinea-pigs tested contains 0·1 Unit or more of tetanus antitoxin per millilitre. Alternatively, the serum of each of not fewer than one-third of the guinea-pigs tested contains 1 Unit or more of tetanus antitoxin per millilitre.

Storage. Tetanus Toxoid should be kept at as low a temperature as possible above the freezing-point and should not be exposed to temperatures exceeding 20°. Under these conditions both forms may be expected to retain their potency for at least two years after the date of manufacture.

Containers. Tetanus Toxoid is distributed in sterilised glass containers sealed so as to exclude bacteria. If more than one dose is included in one container, and if the container allows of the withdrawal of successive doses on different occasions, the Tetanus Toxoid contains a suitable antiseptic in such concentration as will prevent the growth of bacteria.

DOSES

Metric.	Imperial.
By subcutaneous or intramuscular injection.	
0·5 to 1 mil.	8 to 15 minims.

When Tetanus Toxoid is prescribed, Tetanus Toxoid in simple solution shall be dispensed, unless Alum Precipitated Tetanus Toxoid is specified.

UNGUENTUM ACIDI TANNICI

[Ung. Acid. Tann.]

Ointment of Tannic Acid

Synonym. Tannic Acid Ointment.

This Ointment may be made in accordance with the following formula:—

Tannic Acid	200 grammes
Glycerin	200 grammes
Simple Ointment, prepared with Yellow Soft Paraffin . .	600 grammes

Dissolve the Tannic Acid in the Glycerin, and incorporate the solution with the Simple Ointment.

UNGUENTUM AQUOSUM

[Ung. Aquos.]

Hydrous Ointment

Arachis Oil, Cottonseed Oil or Sesame Oil may be used, in place of Olive Oil, in making this Ointment.

UNGUENTUM CAPSICI

[Ung. Capsic.]

Ointment of Capsicum

Synonym. Capsicum Ointment.

Simple Ointment, prepared with Yellow Soft Paraffin, may be used in place of the Lard, Hard Paraffin and Yellow Soft Paraffin, in making this Ointment.

UNGUENTUM HYDRARGYRI COMPOSITUM

[Ung. Hydrarg. Co.]

Compound Ointment of Mercury

Synonym. Compound Mercury Ointment.

Arachis Oil, Cottonseed Oil or Sesame Oil may be used, in place of Olive Oil, in making this Ointment.

APPENDICES

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APPENDICES

APPENDIX IV. G

Addendum, 1936, to the British Pharmacopœia, 1932, page 81,
delete this appendix ;
insert

G. *DETERMINATION OF ULTRA-VIOLET ABSORPTION*

The ultra-violet absorption at a required wave-length is determined by means of a suitable spectrophotometer, the result being calculated as the intensity of absorption ($\text{Log } I_0/I$) of 1 centimetre of a 1 per cent. w/v solution of the substance, where I_0 is the intensity of the incident radiation and I the intensity of the emergent radiation. This value is usually written as $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$

Cod-liver Oil. The ultra-violet absorption at 328 mμ is determined on a solution of the unsaponifiable matter in *dehydrated alcohol* or *cyclohexane*. A preliminary test of a solution of the untreated oil in *cyclohexane* will indicate the quantities of oil and of solvent which will be necessary. The solution of the unsaponifiable matter is prepared as follows :—Boil 1 gramme of the cod-liver oil with 10 millilitres of freshly prepared *N/2 alcoholic solution of potassium hydroxide* for five minutes, or until the solution is clear. Add 20 millilitres of *water*, transfer to a small separator, and extract with two successive quantities of 25 millilitres of *anæsthetic ether*. Wash the mixed ethereal solutions by gentle rotation, without violent shaking, successively with 10 to 20 millilitres of *water*, with 10 to 20 millilitres of *N/2 potassium hydroxide*, and with successive quantities of 10 millilitres of *water*, until the washings are no longer alkaline to *solution* of *phenolphthalein*. Filter the ethereal solution into a flask, wash the filter with *anæsthetic ether*, remove the ether from the mixed filtrate and washings by warming on a water-bath in a current of dry oxygen-free nitrogen, and dissolve the residue in a sufficient quantity of *dehydrated alcohol* or *cyclohexane* to produce a solution of the concentration required for the instrument to be used. The result is calculated with reference to the original oil.

Halibut-liver Oil. The ultra-violet absorption at 328mμ and at 300mμ is determined on a solution of the oil in *cyclohexane*.

Calciferol. The ultra-violet absorption at 265mμ is determined on a solution in *dehydrated alcohol*, special precautions being taken against errors which may be caused by the extreme photo-sensitiveness of Calciferol.

APPENDIX X. C

British Pharmacopœia, 1932, pages 578 and 579,
delete this appendix ;
insert

C. *DETERMINATION OF IODINE VALUE*

The iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance when determined by one of the following methods :—

I. Iodine Monochloride Method. Place the oil, or fat, accurately weighed, in a dry stoppered vessel, add 10 millilitres of *carbon tetrachloride*, and dissolve. Add 20 millilitres of *solution of iodine monochloride*, insert the stopper, previously moistened with *solution of potassium iodide*, and keep in a dark place at a temperature of about 17° for half an hour. Add 15 millilitres of *solution of potassium iodide* and 100 millilitres of *water*; shake, and titrate with *N/10 sodium thiosulphate*, using *mucilage of starch* as indicator. Note the number of millilitres required (*a*). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of millilitres of *N/10 sodium thiosulphate* required (*b*).

Calculate the iodine value from the following formula :—

$$\text{Iodine Value} = \frac{(b - a) \times 0{\cdot}01269 \times 100}{\text{Weight (in grammes) of substance}}.$$

The approximate weight, in grammes, of substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

REAGENT.—*Solution of Iodine Monochloride.* The solution may be prepared by either of the two following methods :—

(1) Dissolve 13 grammes of *iodine* in 1000 millilitres of *glacial acetic acid.* To 20 millilitres of this solution add 15 millilitres of *solution of potassium iodide* and 100 millilitres of *water*, and titrate the solution with *N/10 sodium thiosulphate.* Pass *chlorine*, washed and dried, through the remainder of the iodine solution, until the amount of *N/10 sodium thiosulphate*, required for the titration, is approximately, but not more than, doubled.

(2)	*Iodine Trichloride* . . .	8 grammes
	Iodine	9 grammes
	Glacial Acetic Acid, sufficient to produce	1000 millilitres

Dissolve the *iodine trichloride* in about 200 millilitres of *glacial acetic acid* ; dissolve the *iodine* in about 500 millilitres of *glacial acetic acid* ; mix the two solutions, and add sufficient *glacial acetic acid* to produce the required volume.

Solution of iodine monochloride should be kept in a stoppered bottle, protected from light, and stored in a cool place.

II. Pyridine Bromide Method. Place the substance, accurately weighed, in a dry stoppered vessel, add 10 millilitres of *carbon tetrachloride*, and dissolve. Add 25 millilitres of *solution of pyridine bromide.* Allow to stand for ten minutes in a dark place. Complete the determination as described under *iodine monochloride method* above, beginning with the words "Add 15 millilitres . . .".

The approximate weight in grammes of substance to be taken may be calculated by dividing 12·5 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated on a smaller quantity of the substance being tested.

REAGENT.—*Solution of Pyridine Bromide.* Dissolve 8 grammes of *pyridine* and 10 grammes of *sulphuric acid* in 20 millilitres of *glacial acetic acid*, keeping the mixture cool. Add 8 grammes of *bromine* dissolved in 20 millilitres of *glacial acetic acid* and dilute to 1000 millilitres with *glacial acetic acid.*

Note.—*Glacial acetic acid* of Reagent purity must be used in preparing this reagent.

Iodine Value of the Glycerides of Halibut-liver Oil.—Determine the *iodine value* (*pyridine bromide method*) (x) of the oil. Determine the *unsaponifiable matter* (S) using 1 gramme of the oil, evaporating the acetone from the unsaponifiable matter by means of a current of nitrogen and drying the residue at 80° in a current of nitrogen. Weigh the residue and immediately determine the *iodine value* (*pyridine bromide method*) (y). Calculate the iodine value of the glycerides from the formula

$$\frac{100x - Sy}{100 - S}.$$

APPENDIX X. D

British Pharmacopœia, 1932, page 579,
delete this appendix ;
insert

D. *DETERMINATION OF THE UNSAPONIFIABLE MATTER IN FIXED OILS, AND FATS*

Weigh accurately a quantity of oil or fat, not exceeding 2·5 grammes but not less than 2·0 grammes, and boil on a water-bath for one hour with 25 millilitres of *N/2 alcoholic potassium hydroxide* in a flask provided with a reflux condenser. Wash the contents of the flask into a separator by means of 50 millilitres of *water*, and, while the liquid is still slightly warm, extract by shaking vigorously with three successive quantities of 50 millilitres of *ether*, washing the flask with the first quantity of *ether*. Mix the ethereal solutions in a separator containing 20 millilitres of *water*. (If the ethereal solutions contain solid suspended matter, filter them into the separator through a fat-free filter-paper and wash the filter-paper with *ether*.) Gently rotate the separator for a few minutes without violent shaking, allow the liquids to separate, and run off the aqueous layer. Wash the ethereal solution by shaking vigorously with two successive quantities of 20 millilitres of *water*; then treat three times with 20 millilitres of *N/2 aqueous potassium hydroxide*, shaking vigorously on each occasion, each treatment being followed by washing with 20 millilitres of *water*. Finally wash with successive quantities of 20 millilitres of *water* until the aqueous layer is no longer alkaline to *solution of phenolphthalein*. Transfer the ethereal extract to a weighed flask, washing out the separator with *ether*; distil off the ether and add 3 millilitres of *acetone*. By the aid of a gentle current of air remove the solvent completely from the flask, which is preferably held obliquely and rotated, almost entirely immersed in boiling water. Dry to constant weight at a temperature not exceeding 80° and dissolve the contents of the flask in 10 millilitres of freshly-boiled *alcohol* (*95 per cent.*), previously neutralised to *solution of phenolphthalein*. Titrate with *N/10 sodium hydroxide*, using *solution of phenolphthalein* as indicator. If the amount of *N/10 sodium hydroxide* required does not exceed 0·1 millilitre, the amount weighed is to be taken as the unsaponifiable matter. Calculate the unsaponifiable matter as a percentage of the oil or fat. If the amount of *N/10 sodium hydroxide* required exceeds 0·1 millilitre, the amount weighed cannot be taken as the unsaponifiable matter, and the test must be repeated.

Reagent.—*Solution of Potassium Hydroxide, Alcoholic, N/2*: A solution containing 28·05 grammes KOH in 1000 millilitres of *alcohol (95 per cent.)*.

APPENDIX XI. C

DETERMINATION OF ALDEHYDES IN VOLATILE OILS

British Pharmacopœia, 1932, page 582, after line 30, *insert*

(3) Purified Volatile Oil of Bitter Almond.

Carry out the process described for Oil of Lemon, using about 0·5 gramme, accurately weighed, of the Purified Volatile Oil of Bitter Almond, with 12 millilitres of *hydroxylamine hydrochloride reagent in alcohol (60 per cent.)*. Each millilitre of *N/2 potassium hydroxide* is equivalent to 0·05345 gramme of C_6H_5CHO.

APPENDIX XV. O

ASSAY OF VITAMIN A

4. Suggested Details of Spectrophotometric Method, Addendum, 1936, to the British Pharmacopœia, 1932, pages 89–91,

delete this section;
insert

4. The Spectrophotometric Method.

The ultra-violet absorption at 328mμ is determined by the method of Appendix IV. G. An expression of the content of vitamin A in Units per gramme is obtained by multiplying the *ultra-violet absorption* by the factor declared by the Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations as the factor to be used for this purpose.[1]

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[1] The factor accepted at present (April, 1940) is 1600.

The vitamin A content which has been obtained in this way should be accompanied by a statement indicating the method of assay employed.

The spectrophotometric method, as described, measures the amount of a substance having a certain physical property characteristic of vitamin A. When applied to the determination of vitamin A in a specimen of cod-liver oil or halibut-liver oil which conforms in all other respects to the Pharmacopœial requirements, it gives a trustworthy measurement, but it may be inapplicable in the presence of other substances showing absorption in the region of 328mμ. In the event of a discrepancy, due to this or any other cause, between the Units of vitamin A in a preparation of vitamin A, as determined by the biological method and by the spectrophotometric method respectively, the value as determined by the biological method shall be accepted.

Limits of Error :—The limits of error (P = 0·99) for the actual physical measurement of the intensity of absorption at 328mμ depend on the level of absorption and the number of replicate tests made.

The following table gives the values obtained under different conditions :—

Intensity of absorption $E^{1 \text{ per cent.}}_{1 \text{ cm.}}$	Single tests per cent.	Tests in duplicate per cent.	Tests in quadruplicate per cent.
0·33	80 and 120	86 and 114	90 and 110
0·67	90 and 110	93 and 107	95 and 105
1·33	95 and 105	96·5 and 103·5	97·5 and 102·5

No information is available for the calculation of the error of the factor.

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Synonyms appear with cross references.
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